Original Research

Association between serum placental growth factor and vascular endothelial function in hyprtensive disorders complicating pregnancy

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Impact statement

HDCP treatment is limited and mainly focuses on controlling acute hypertension, preventing eclampsia, and timely delivery. Therefore, the prevention and prediction of hypertension during pregnancy are essential. This study measured the levels of serum PLGF, as well as plasma ET-1 and NO, between the HDCP patients and the normal pregnancy controls. In addition, the vascular endothelial function of pregnant women was analyzed by the ET technique. The levels of serum PLGF were positively correlated with plasma NO levels and negatively correlated with the levels of plasma ET-1. Furthermore, the vascular endothelial function of the patients with HDCP correlated with their levels of PLGF. Serum PLGF was negatively correlated with the values of EP, β, PWVβ, and AI, whereas positively correlated with the value of AC. Therefore, the association between serum PLGF and vascular endothelial function in HDCP was expected to predict gestational hypertension accurately.

Abstract

Hypertensive disorders complicating pregnancy (HDCP) is a systemic disease among pregnant women. Therefore, the prevention and prediction of hypertension during pregnancy are critical. This study aimed to clarify whether the vascular endothelial function of women with gestational hypertension was linked to placental growth factor. A total of 200 pregnant women were enrolled in our study and subsequently divided into two groups: the HDCP group and the normal pregnancy controls. The levels of serum placental growth factor, as well as plasma endothelin-1 and nitric oxide, between the two groups were measured. In addition, the endothelial function indexes, including pressure-strain elasticity coefficient (EP), the common carotid stiffness index (β), arterial compliance, single-point pulsed-wave velocity, and augment index (AI) of bilateral common carotid arteries, were compared between the HDCP and control groups using the echo tracking technique. In our study, the level of placental growth factor in the HDCP group was significantly lower than the control group. Furthermore, our results clarified that endothelin-1 increased while nitric oxide decreased in the HDCP group compared with the control group. On the other hand, we found that EP, β, pulsed-wave velocity and augment index values were significantly higher in the HDCP group than in the control group ($P < 0.001$). However, the value of arterial compliance was significantly decreased in patients of the HDCP group compared with the control group ($P < 0.001$). In conclusion, the association between serum placental growth factor and vascular endothelial function in HDCP could serve as a more accurate predictive factor of pregnant hypertension.

Keywords: PLGF, HDCP, echo tracking, ET-1, NO

Experimental Biology and Medicine **2022; 247: 480–487. DOI: 10.1177/15353702211063833**

Introduction

Hypertensive disorders complicating pregnancy (HDCP) is a systemic disease among pregnant women. The main clinical features usually manifest as edema, hypertension, and proteinuria in pregnant women with onset from 20 weeks of gestation to two weeks postpartum.[1,2] In China, HDCP occurs in 9.4% to 10.4% of pregnancies and contributes significantly to maternal and perinatal morbidity and mortality.[3,4] Increased systemic vascular resistance in pregnant women has an adverse impact on the blood flow of many organs.[5] Thus, it is a severe threat to maternal and fetal health, including preterm birth, perinatal death, renal or

hepatic failure, disseminated intravascular coagulation, stroke, and even death to the pregnant women and the fetus.[6]

As a secretory glycosylated homodimer, the placental growth factor (PLGF) is a member of the vascular endothelial growth factor (VEGF) family.[7] The serum level of PLGF secretion in normal pregnant women shows a bell shape: the level of PLGF is low when the embryo is in a hypoxia state during growth and development; the level of PLGF increases when the blood flow in the placental unit of the fetus is established at 10 to 12 weeks of gestation, and it reaches the peak at 28 to 30 weeks of gestation.[8] The level of PLGF in pregnant women with severe preeclampsia in the third trimester of pregnancy is significantly lower than the normal pregnancy group.[9] Decreased PLGF levels in the first trimester of pregnancy could also lead to imbalanced placental angiogenesis, resulting in preeclampsia in the second or third trimester of pregnancy.[10] PLGF significantly affects angiogenesis, integrity, and vascular wall permeability, obstructing placental vascular construction.[11,12]

Echo tracking (ET) technique is an ultrasound imaging diagnostic technique for evaluating vascular elasticity. It has been applied in clinical ultrasound technology in recent years. This technology could dynamically track and trace the movement of the arterial wall and display the change curve of pipe diameter, automatically calculate and analyze vascular elasticity index, and then detect atherosclerosis change before vascular morphology and structure change.[13] It is ideal for detecting early vascular lesions due to its advantages, including high quality, simple operation, non-invasive nature, and good reproducibility.[13] A previous study showed that carotid arterial stiffness was greater in women with preeclampsia, and the ET technique could non-invasively identify changes in vascular elasticity in women with preeclampsia.[14,15]

However, the mechanism underlying HDCP has not been fully understood. HDCP treatment is limited and mainly focuses on controlling acute hypertension, preventing eclampsia, and timely delivery. Therefore, the prevention and prediction of hypertension during pregnancy are essential. This study aimed to clarify whether the vascular endothelial function of women with gestational hypertension (GH) was linked to PLGF. Combining the two indexes was expected to predict the HDCP more accurately and provide a novel preventive and therapeutic approach.

Materials and methods

Study population

All participants should meet the inclusion criteria: (1) over 18 years of age; (2) singleton pregnancy; (3) no chronic medical diseases or other pregnancy complications.

Diagnostic criteria of HDCP: patients should meet the diagnostic criteria for HDCP published by the American College of Obstetricians and Gynecologists on guidelines for hypertension during pregnancy in 2013.[16] Diagnostic criteria of GH: (1) patients were with normal blood pressure (BP) previously; (2) after 20 weeks of gestation, patients were with systolic BP $\geq$140 mmHg or diastolic BP $\geq$90 mmHg at 4-h interval. Diagnostic criteria for preeclampsia: (1) hypertension as defined above; (2) urinary protein $\geq$0.3 g/day or urine protein/creatinine $\geq$0.3. Diagnostic criteria of severe preeclampsia: (1) systolic BP $\geq$160 mmHg or diastolic BP $\geq$110 mmHg; (2) urinary protein $\geq$5 g per 24 h; (3) accompanied by thrombocytopenia, liver or renal function impairment, pulmonary edema, and cerebral or visual disorders.

According to the inclusion criteria, 200 pregnant women were enrolled in our study. They were divided into the HDCP group (n = 120) and the normal pregnancy controls (n = 80). The HDCP group met the diagnostic criteria of HDCP and was enrolled at the gestational week of 35.27 ± 3.08. The normal pregnancy controls included pregnant women without any complications, and they were enrolled at the matched gestational week of 34.89 ± 2.53. Physical examinations were performed for all participants, and basic demographic characteristics were recorded, including age, gestational week, body mass index (BMI), and medical history (e.g. hypertension and diabetes history). Our research was fully approved by the Ethics Committee of Shijiazhuang Fourth Hospital, and all participants agreed to participate being informed of the research content and signing informed consent.

Laboratory procedures

We obtained 5 mL overnight fasting venous blood samples (no more than 12 h) from each participant to detect the levels of serum PLGF, endothelin-1 (ET-1), nitric oxide (NO). All the blood samples were collected via venipuncture into ethylene diamine tetraacetic acid-vacutainer (BD Vacutainer®, USA) and centrifuged as soon as possible.

The Alere Triage PLGF fluorescence immune analyzer (Alere, Inc., San Diego, USA) was used to measure the level of serum PLGF. It used double-antibody sandwich fluorescence immunoassay, and the operation strictly followed the instructions. First, a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) was conducted to quantify the amount of ET-1 according to the manufacturer's instructions (East Asia Immune Technology Co. Ltd, catalog no. 9603/9604, Beijing). Second, nitrate reductase activity assay was used to detect plasma NO according to the manufacturer's instructions (Bluegene Biotech Co., Ltd, catalog no.180713, Shanghai).

Ultrasound examinations and ET analysis

Pro-sound SSD A10 Premier Ultrasound Machine (Aloka Co, Ltd, Tokyo, Japan) was used to measure pressure-strain elasticity coefficient (EP), common carotid stiffness index (β), arterial compliance (AC), single-point pulsed-wave velocity (PWVβ), and augment index (AI) of bilateral common carotid arteries. In addition, the machine was also equipped with a UST-5411 linear Array to transduce (13 MHz), ET analysis software, and an extended data-management subsystem (E-DMS).

All participants were breathing calmly and in a supine occipital position. The long axis of the common carotid artery was taken for an ultrasound examination. The direction was adjusted to avoid the jugular vein. ET

software was activated to record each image after more than 10 stable cardiac cycles. During the analysis, more than five baseline stable waveforms were recorded. All the indexes were calculated automatically.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) Version 18.0 software (Armonk, NY) and GraphPad Prism version 5.01 (San Diego, CA) were used for data analysis and calculation. The measurement data were presented as mean ± standard deviation (SD). Unpaired Student's t-test was performed for comparisons between two groups. One-way analysis of variance (ANOVA) was used to determine comparisons among multiple groups. Pearson's correlation was performed to analyze the correlation between variables. $P < 0.05$ was considered statistically significant.

Table 1. Clinical characteristics of pregnant patients with hypertensive disorders complicating pregnancy (HDCP) and normal pregnancy (Control).

Variables	Control (n = 80)	HDCP (n = 120)	P
Maternal age (years)	27.37 ± 2.93	29.81 ± 3.36	0.084
Gestational weeks at diagnosis	34.89 ± 2.53	35.27 ± 3.08	0.436
BMI before pregnancy (kg/m^2)	21.51 ± 2.48	24.93 ± 4.11	0.017
Enrolled BMI (kg/m^2)	27.46 ± 3.32	33.17 ± 4.76	0.003
Proteinuria (mg/24 h)	79.64 ± 47.18	273.89 ± 87.35	<0.001
SBP (mmHg)	117.38 ± 7.79	149.57 ± 9.28	<0.001
DBP (mmHg)	79.14 ± 6.53	101.38 ± 8.16	<0.001
Clinical classification			
GH	–	56 (46.67%)	–
mPE	–	43 (35.83 %)	–
sPE	–	21 (17.5 %)	–

Note: The data presented are mean ± SD or n (percentage). The comparison of data between the two group was done by unpaired t test.
BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; GH: gestational hypertension; mPE: mild preeclampsia; sPE: severe preeclampsia.

Results

PLGF level of HDCP group was lower than that of the control group

Table 1 shows the basic demographic characteristics of 120 HDCP patients and 80 normal pregnancy controls. No significant differences were found in terms of age and gestational weeks. However, there were significant differences between BMI before pregnancy and after enrollment ($P = 0.003$). This is an apparent phenomenon, as after pregnancy, women gain considerable weight, but their height does not change. Among patients with HDCP, we found that the Pearson correlation coefficient between BMI and NO was 0.037 ($P = 0.638$), and the Pearson correlation coefficient between BMI and ET-1 was 0.023 ($P = 0.481$), indicating that BMI had no significant effect on vascular endothelial function in the present study. According to the diagnostic criteria, HDCP patients were divided into three subgroups: GH), mild preeclampsia (mPE), and severe preeclampsia (sPE) group. The number of each group is shown in Table 1.

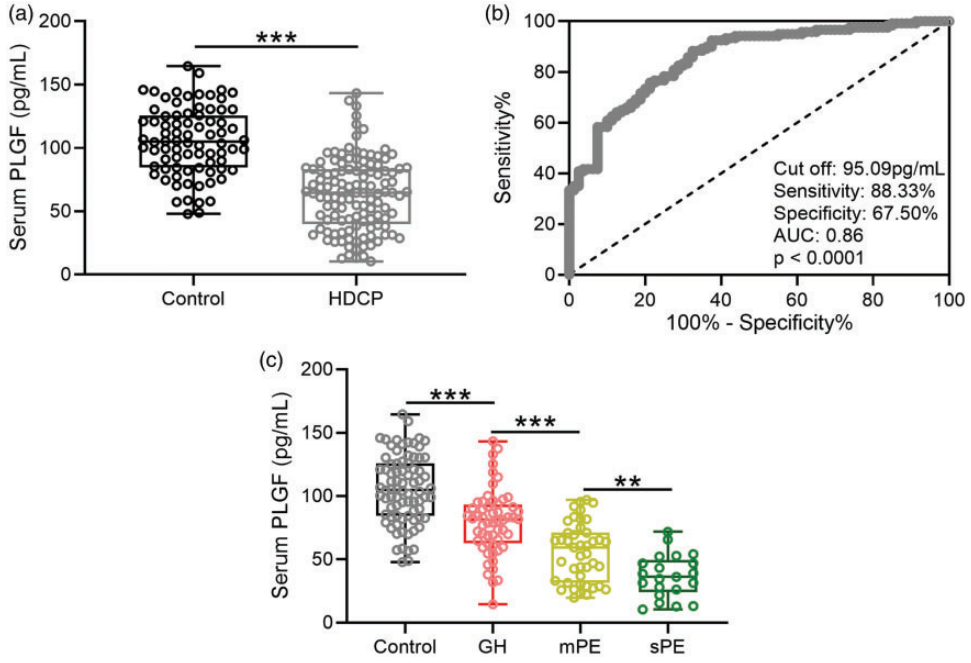

Figure 1. (a) Comparison of serum PLGF between patients with HDCP and normal pregnancy (Control). ***$P < 0.001$, unpaired Student's t-test. (b) ROC analysis of serum PLGF on hypertensive disorders complicating pregnancy from normal pregnancy. (c) Comparison of serum PLGF among normal pregnancy, gestational hypertension, mild preeclampsia, and severe preeclampsia groups. **$P < 0.01$, ***$P < 0.001$, One-way ANOVA followed by a Dunn's multiple comparisons test. (A color version of this figure is available in the online journal.)
GH: gestational hypertension; mPE: mild preeclampsia; sPE: severe preeclampsia; HDCP: hypertensive disorders complicating pregnancy; PLGF: placental growth factor.

We tested the serum PLGF levels of all pregnant women. The serum PLGF between the control group and the HDCP group and among the three subgroups of the HDCP group were consistent with the previous study.[9] However, PLGF levels of pregnant women in the HDCP group were significantly lower than those in the control group ($P < 0.001$) (Figure 1(a)). In addition, the levels of PLGF significantly and gradually decreased among the GH group, the mPE group, and the sPE group (Figure 1(c)) ($P < 0.01$). Figure 1 (b) shows the receiver operating characteristic (ROC) curves of serum PLGF on HDCP of the normal pregnancy control group.

Reduced NO and increased ET-1 in HDCP group

The relationship among serum PLGF, plasma ET-1 and NO is presented in Figure 2. ET-1 and NO were two representative indicators of endothelial function. The levels of plasma NO were significantly lower in the HDCP group than in the control group, while the levels of plasma ET-1 were significantly higher in the HDCP group (Figure 2(a) and (b)). Moreover, the levels of NO decreased, whereas ET-1 levels increased gradually and significantly among the GH group, the mPE group, and the sPE group (Figure 2(c) and (d)) ($P < 0.01$). In addition, the levels of serum PLGF were positively correlated with the levels of plasma NO and negatively correlated with the levels of plasma ET-1 (Figure 2(e) and (f)).

Comparison of endothelial function between the HDCP and control group

The endothelial function indexes between the HDCP and control groups, including EP, β, AC, PWVβ, and AI of bilateral common carotid arteries, were compared using the ET analysis (Figure 3(a) to (e)). We found that EP, β, PWVβ, and AI values were significantly higher in the HDCP group than in the control group ($P < 0.001$). However, the value of AC was significantly decreased in the patients of the HDCP group compared with the control group ($P < 0.001$).

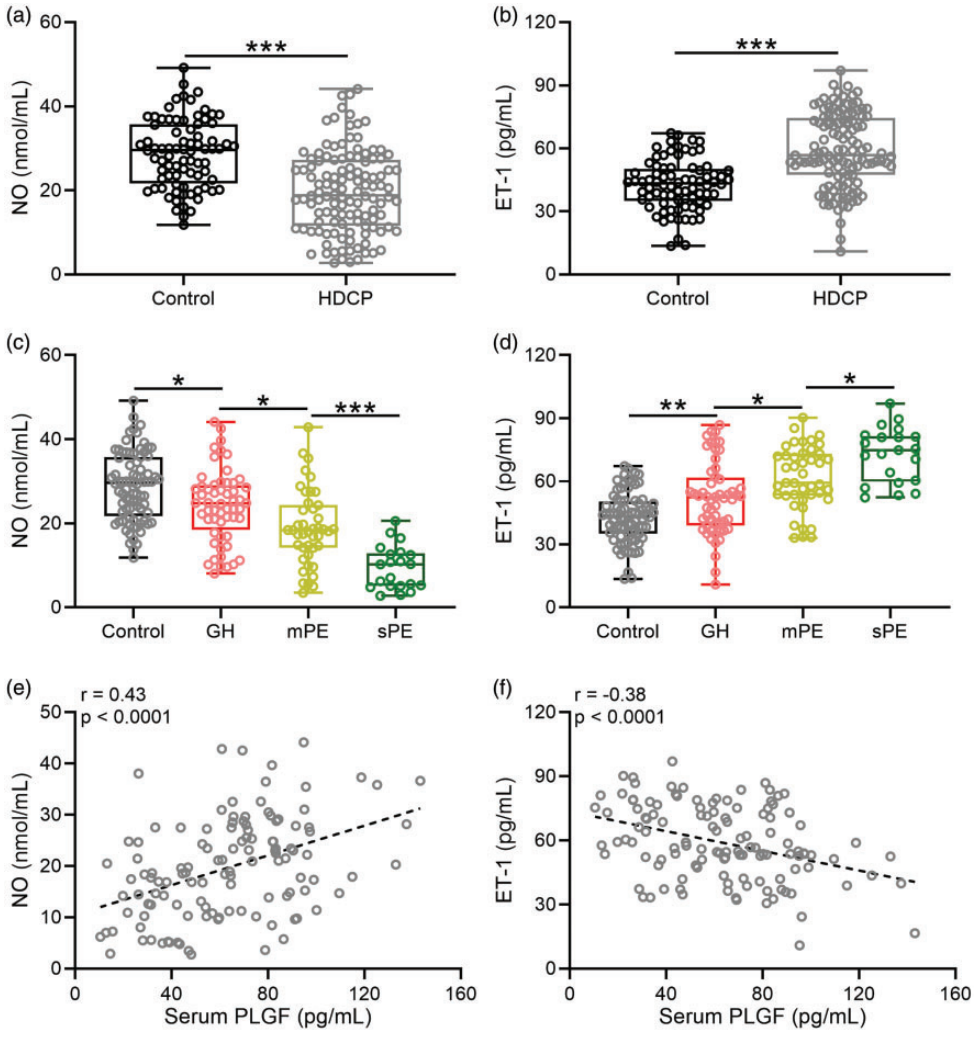

Figure 2. Comparison of plasma NO (a) and ET-1 (b) between patients with hypertensive disorders complicating pregnancy (HDCP) and normal pregnancy (Control). ***$P < 0.001$, unpaired Student's t-test. Comparison of plasma NO (c) and ET-1 (d) among normal pregnancy, gestational hypertension, mild preeclampsia, and severe preeclampsia groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, One-way ANOVA followed by a Dunn's multiple comparisons test. Pearson's correlation analysis was carried out to measure the correlations between serum PLGF and plasma NO (e), ET-1 (f) from patients with hypertensive disorders complicating pregnancy (HDCP, $n = 120$). (A color version of this figure is available in the online journal.)
ET-1: endothelin-1; PLGF: placental growth factor; GH: gestational hypertension; mPE: mild preeclampsia; sPE: severe preeclampsia; PLGF: placental growth factor.

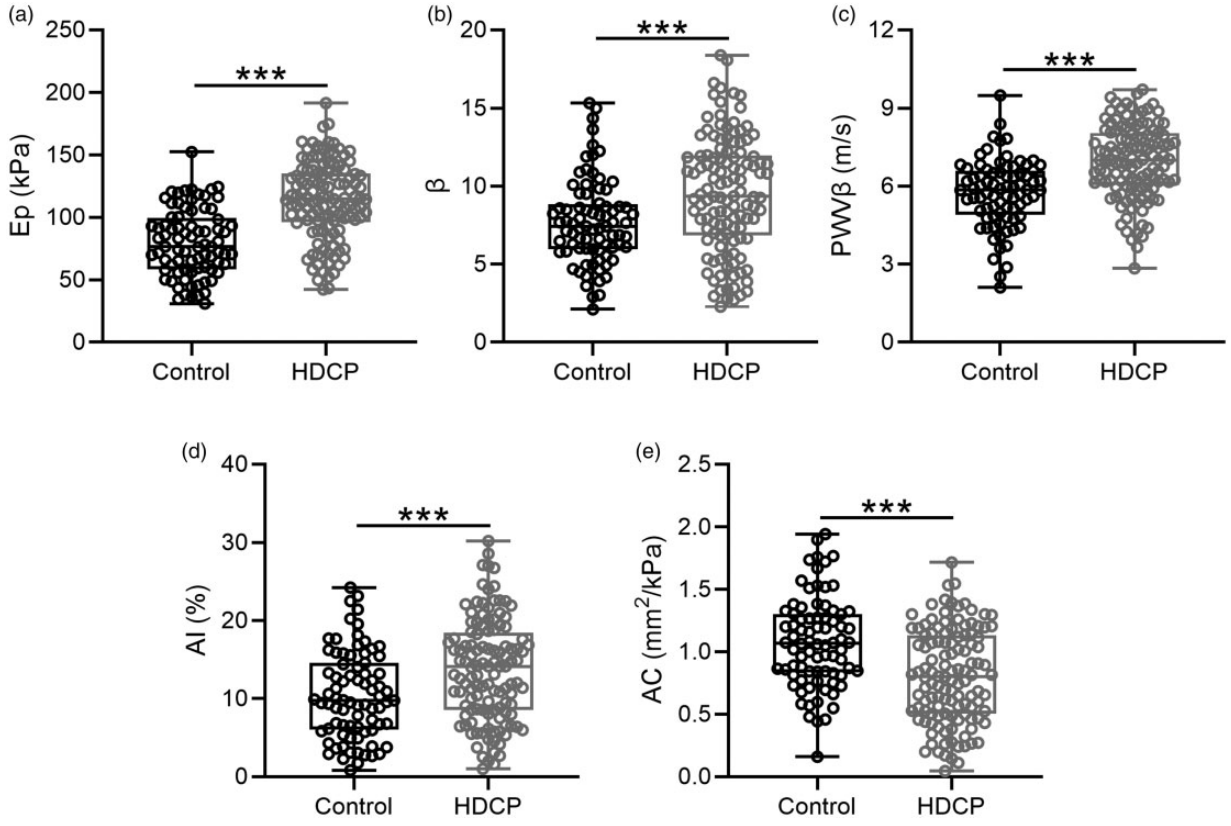

Figure 3. Comparison of pressure-strain elasticity coefficient (EP, a), the common carotid stiffness index (β, b), single-point pulsed wave velocity (PWVβ, c), augment index (AI, d), and arterial compliance (AC, e) between patients with hypertensive disorders complicating pregnancy (HDCP) and normal pregnancy (Control).
***$P < 0.001$, unpaired Student's t-test.

PLGF level was correlated with vascular endothelial function

We then performed Pearson's correlation analysis to clarify the correlation between serum PLGF and ET parameters in patients of the HDCP group (Figure 4). The result showed that the levels of serum PLGF were negatively correlated with the values of EP, β, PWVβ, and AI (Figure 4(a) to (d)), whereas positively correlated with AC (Figure 4(e), $P < 0.001$). These are consistent with our hypothesis that decreased serum PLGF was associated with vascular endothelial injury.

AC stands for the compliance of the common carotid artery, which decreases with changes in vascular morphology and structure, and destruction of elastic fibers. It was consistent with the declining trend of vascular endothelial function in HDCP patients. Therefore, AC parameters of HDCP patients decreased and positively correlated with serum PLGF. However, we did not find a similar association in the control group (serum PLGF correlation with EP: $R = -0.18$, $P = 0.319$; correlation with β: $r = -0.09$, $P = 0.642$; correlation with PWVβ: $R = -0.13$, $P = 0.258$; correlation with AI: $r = -0.16$, $P = 0.225$; correlation with AC: $R = 0.11$, $P = 0.317$).

Discussion

HDCP is known as a pervasive pregnancy-specific disease in obstetrics. Previous studies have indicated that pregnant women with PE could have systematic vasospasm, which leads to hemoconcentration and increases peripheral vascular resistance.[4] Furthermore, HDCP could even cause death to pregnant women and fetuses.[6] Therefore, it is crucial to explore the pathogenesis of hypertension during pregnancy for early detection and prevention of HDCP to increase the life quality of pregnant women and fetuses. This study measured serum PLGF and plasma ET-1 and NO levels between the HDCP patients and the normal pregnancy controls. In addition, the vascular endothelial function of pregnant women was analyzed by the ET technique. We also examined the correlation between the ET parameters and the levels of serum PLGF and plasma ET-1 and NO.

As a member of the VEGF family, PLGF is mainly expressed in the placenta. The human gene is located on chromosome 14q14. Soluble fms-like-tyrosine-kinase 1 (sFLT1) has a high affinity with PLGF and can change the permeability and integrity of the vascular wall by reducing and inhibiting VEGF and PLGF activity in vascular formation disorders.[17] Previous studies have demonstrated that the levels of PLGF in pregnant women are linked with the development of the placenta, pregnancy, and preeclampsia.[18] PLGF knockout mice showed abnormal placental blood vessels at the implantation site.[19] In the first trimester of pregnancy, the level of PLGF remains low and then increases from the 11th to 12th week of gestation, eventually reaching a peak at the 30th week, and falls

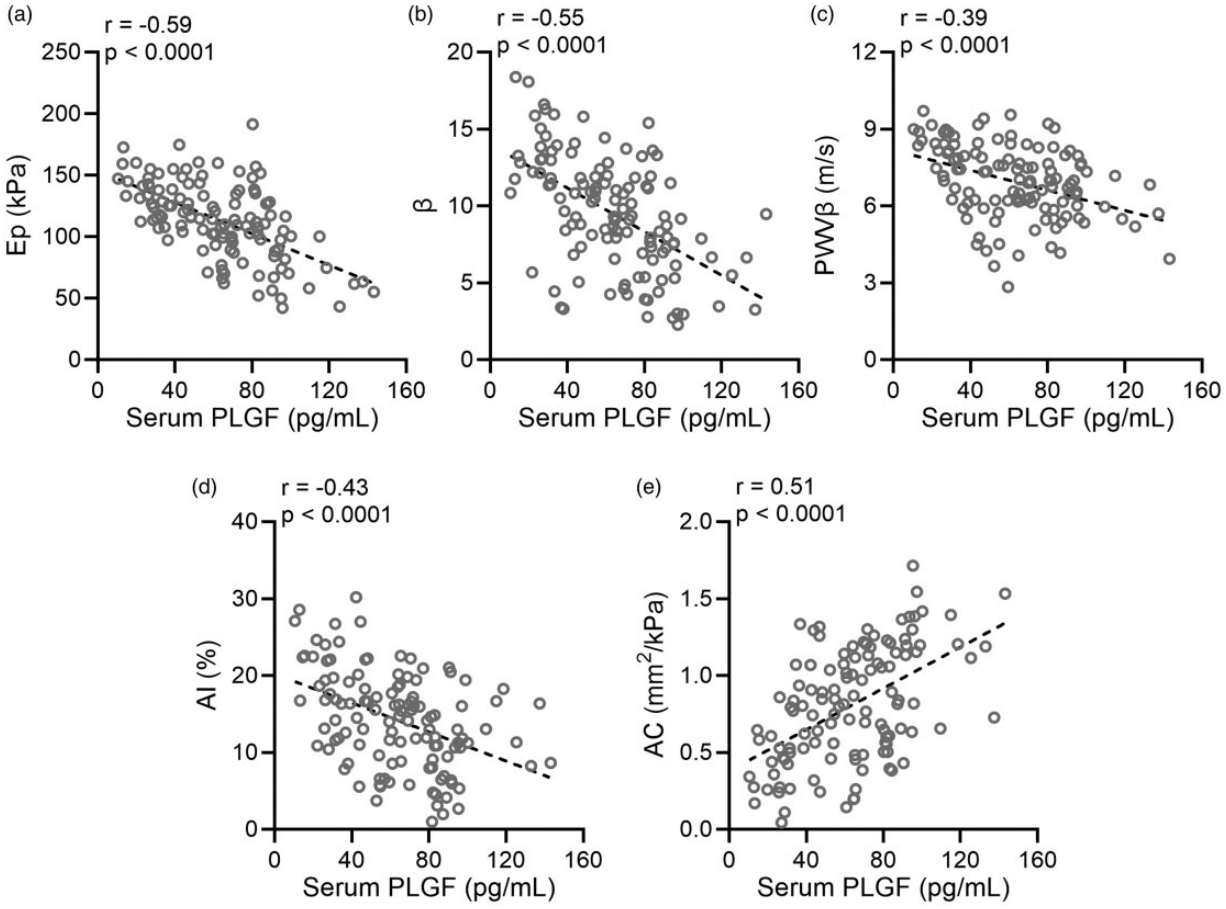

Figure 4. Pearson's correlation analysis was carried out to measure the correlations between serum PLGF and pressure-strain elasticity coefficient (EP, a), the common carotid stiffness index (β, b), single-point pulsed wave velocity (PWVβ, c), augment index (AI, d), and arterial compliance (AC, e) from patients with hypertensive disorders complicating pregnancy (HDCP, n = 120).
PLGF: placental growth factor.

subsequently.[8] However, in preeclampsia patients, the serum and urinary PLGF levels are decreased at the time of diagnosis.[7] In the third trimester of pregnancy, lower PLGF levels are significantly more prevalent than normal pregnant women.[8] Decreased PLGF levels in the first trimester of pregnancy could also lead to imbalanced placental angiogenesis, resulting in preeclampsia in the second or third trimester of pregnancy.[10] In our study, the levels of PLGF in the HDCP group were significantly lower than in the control group, consistent with the above previous studies.

ET-1 and NO, as representative indicators of endothelial function, are both produced by the vascular endothelial cells.[20] NO is synthesized by L-arginine through endothelial NO synthetase and plays an influential role in vasodilation and anti-coagulation. N-monomethyl-L-arginine (L-NMMA) is an analog of L-arginine and could inhibit NO formation, leading to hypertension.[21] Previous studies found that experimental rats during early gestation infused with L-NMMA subcutaneously showed symptoms similar to preeclampsia.[22] Therefore, abnormalities in NO production by endothelial cells may play a role in the vascular physiology of preeclampsia. The concentrations of serum NO metabolites have been significantly lower in the sPE patients than in the healthy pregnant women.[4]

On the other hand, ET-1, a potent vasoconstrictor peptide, is secreted by endothelial cells and involved in smooth muscle cell proliferation and vasoconstriction. Therefore, ET-1 was thought to account for the maintenance of BP and impact the response of endothelial cells damage.[20] The pathogenesis of hypertension is also linked to ET-1.[23] It is reported that plasma levels of ET-1 were increased in preeclampsia patients,[24] where the release of ET-1 in the blood could lead to systemic vasospasm and increased renal vascular resistance, while hypertension enhanced ET-1 synthesis through damaging the endothelial structure.[24] ET-1 is also reported to induce a hypertensive response in vascular smooth muscle cells and NO-mediated hypotensive response from endothelial cells.[25] In addition, increased NO synthesis could inhibit the production of ET-1, while decreased NO synthesis enhances ET-1 production,[20] suggesting a synergistic effect between ET-1 and NO could mediate endothelial function. Our results clarified that serum ET-1 and NO levels exhibited a similar change trend as reported in previous studies, where ET-1 increased and NO decreased in the HDCP group compared with the control group. These results demonstrate that the levels of serum PLGF, plasma ET-1, and NO were correlated with HDCP. Serum PLGF was positively correlated with plasma NO and negatively correlated

with plasma ET-1. In addition, serum PLGF was negatively correlated with the values of EP, β, PWVβ, and AI, whereas positively correlated with AC.

Then, we used the ET technique to analyze the compliance and stiffness in the common carotid artery of the participants to verify their vascular endothelial function. ET technique evaluates vascular elasticity through the expansion changes of arterial inner diameter during systole and diastole. The parameters EP, β, AC, PWVβ, and AI were compared between the HDCP and control groups, where EP, β, PWVβ, and AI values were significantly higher in the HDCP group, suggesting that the carotid artery of the HDCP group was more susceptible to damage. In addition, the value of AC was significantly decreased in patients of the HDCP group compared with the control group. As decreased AC indicates changed vascular morphology and destruction of elastic fibers, lower AC values in the HDCP group indicated potential damages in the carotid artery.

Finally, we clarified the correlation between serum PLGF and ET parameters in patients of the HDCP group. Our results showed that serum PLGF was negatively correlated with EP, β, PWVβ, and AI values, while positively correlated with AC. This result indicated that the vascular endothelial function of the HDCP patients correlated with their PLGF levels. Therefore, the association between serum PLGF and vascular endothelial function in HDCP is a potential indicator to predict GH accurately.

Several limitations exist in our study. Firstly, the study recruited a relatively small sample size and selection, only 200 in total in one hospital. Secondly, antihypertensive medication was not considered in our study. Finally, the ET technique relied on many aspects, including the operator's technique, patient's respiratory status, and image quality. Therefore, stability and reproducibility need to be further improved. Although these limitations may affect our result, the association between serum PLGF and vascular endothelial function during HDCP could be helpful for early diagnosis of the HDCP. At the same time, further study on the etiology and pathogenesis of HDCP may provide a novel approach for its prevention and treatment, thereby reducing the maternal mortality rate and improving the prognosis of offspring.

In conclusion, the level of PLGF and vascular endothelial function indicators, ET-1, and NO, were correlated with HDCP. In addition, the vascular endothelial function of the HDCP patients correlated with their PLGF levels. Therefore, the association between serum PLGF and vascular endothelial function in HDCP could serve as a more accurate indicator for predicting pregnant hypertension.

AUTHORS' CONTRIBUTIONS

JZ designed and supervised the study. SL, YL, WW, YD, LQ, and JR performed experiments and analyzed the data. WL mainly wrote the article. JZ and WL wrote the article revisions. All authors reviewed the results and approved the final version of the article

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

Our research was fully approved by the Ethics Committee of Shijiazhuang fourth hospital, and all participants agreed to participate by informing the research content and signing informed consent.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was supported by Hebei Provincial Health Commission Youth Science and Technology Project (20191442).

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(Received August 2, 2021, Accepted November 6, 2021)